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Standardization of unani compound formulation *Safoof-e-musakkin*

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Abstract
Safoof-e-Musakkin is a traditional Unani polyherbal formulation used to treat neurological disorders, particularly anxiety, headache, and delirium. The formulation consists of *Beekh-e-Asrol* (Roots of *Rauvolfia serpentina*), *Kasneez Khushk* (Seeds of *Coriandrum sativum*), and *Filfil-e-Siyah* (fruits of *Piper nigrum*). The primary objective of this study was to standardize Safoof-e-Musakkin using organoleptic, physicochemical, and phytochemical analyses. The ingredients were sourced, cleaned, and shade-dried before being powdered through a sieve (No. 80). The powders were mixed according to the National Formulary of Unani Medicine. Standardization was carried out through organoleptic evaluation, various physicochemical tests including total ash value, water-soluble ash, acid-insoluble ash, and loss of weight on drying at 105 °C. Phytochemical screening and Thin Layer Chromatography fingerprinting were also performed. The organoleptic evaluation of Safoof-e-Musakkin revealed a light brown color, characteristic odor, bitter taste, and moderately fine texture. Physicochemical analysis showed a total ash value of 12.5 ± 0.3%, water-soluble ash of 3.6±0.4%, acid-insoluble ash less than 0.2% and loss of weight on drying at 105 °C was 7.1±0.6%. Phytochemical screening identified the presence of tannins, phenols, flavonoids, terpenoids, saponins, carbohydrates, and reducing sugars. Thin Layer Chromatography fingerprinting analysis was performed on Safoof-e-Musakkin under 254 nm and 366 nm UV light. Vanillin-sulphate reagent was used for visualization, and the plate was heated at 110 °C for 5 minutes. Safoof-e-Musakkin was successfully standardized through comprehensive organoleptic, physicochemical, phytochemical analysis and Thin Layer Chromatography fingerprinting. The data obtained serves as a reference standard for future quality control and standardization of this formulation.

Keywords: Neurological disorders, physicochemical, phytochemical, Safoof-e-musakkin, standardization

1. Introduction
Traditional remedies have long been an integral part of modern pharmaceutical science, with numerous bioactive ingredients derived from natural products playing a significant role in the discovery of synthetic medicines. The Unani, Ayurveda, and Siddha systems of medicine, among others, utilize raw materials sourced primarily from plants, animals, and minerals in the preparation of medicinal formulations. Research has demonstrated the profound effects of crude plant extracts in disease treatment, often showing superior efficacy compared to isolated active ingredients, likely due to their synergistic actions. Unlike synthetic drugs, which, despite their specific therapeutic effects, often pose undesirable side effects, phytochemicals in plant-based medicines generally exhibit lower toxicity, making them an attractive option in medical science. The phytochemical constituents of medicinal plants are known to enhance the body's defense mechanisms, providing protection against a wide range of diseases. In recent years, plant-based medicines have gained popularity due to their lower cost, reduced side effects, and greater effectiveness compared to conventional synthetic drugs. This underscores the significant role of active phytochemicals in addressing various health issues.

In the Unani system of medicine, numerous single (*Mufrad*) and compound (*Murakkab*) herbal preparations are utilized for the treatment of various diseases. Among these, Safoof-e-Musakkin is a well-known poly-herbal formulation used to treat neurological disorders, particularly anxiety [1] headaches, and delirium [2]. This formulation is believed to have a sedative effect on nerve conduction (*Musakkin-e-Asab*), contributing to its efficacy in managing these conditions. Safoof-e-Musakkin comprises *Beekh-e-Asrol* (*Rauvolfia serpentina*), *Kasneez Khushk* (*Coriandrum sativum*), and *Filfil-e-Siyah* (*Piper nigrum*), all of

which are readily available in Sri Lanka, making it feasible to prepare and use locally for treating anxiety, headaches, and delirium [2].

However, despite its traditional use, Safoof-e-Musakkin has not yet undergone thorough physicochemical and phytochemical standardization in Sri Lanka. Standardization is essential to assess the quality, purity, and efficacy of this formulation [3]. Therefore, this study aims to establish quality control standards for Safoof-e-Musakkin using scientific analytical methods, ensuring the formulation's consistency and reliability for therapeutic use.

Table 1: Ingredients of Safoof-e-Musakkin

No	Urudu Name	Botanical Name	Family	Sinhala Name	Part Used
1	*Asrol*	*Rauvolfia serpentina*	Apocynaceae	*Ekaweriya*	Root
2	*Kasneez khushk*	*Coriandrum sativum*	Umbelliferae	*Kottamalli*	Seed
3	*Filfil-e-siyah*	*Piper nigrum*	Piperaceae	*Gammiris*	Fruit

Rauvolfia serpentina (commonly known as snakeroot or windy root) belongs to the Apocynaceae family. In Tamil, it is referred to as *Chivan Amalpodi*, and in Sinhala, it is called *Ekaweriya*. The root of *Rauvolfia serpentina* contains several alkaloids, including serpentinine, ajmaline, ajmalacine, rauwolfine, and rauwolscine [4]. *Rauvolfia serpentina* exhibits various therapeutic properties, including *Musakkin* (sedative), *Mukhadir* (numbing), *Musakkin e Asab* (nerve conduction sedative), and *Munawwim* (hypnotic). These properties contribute to its effectiveness in treating conditions such as Generalized Anxiety Disorder (GAD) [1], headaches, and delirium [5]. *Rauvolfia serpentina* has cardiovascular benefits, including the reduction of heart rate and the dilation of blood vessels, leading to a decrease in blood pressure. It is commonly used to treat insomnia, irritative conditions of the nervous system, and mental symptoms associated with hypertension [6].

Coriandrum sativum (commonly known as *Khasneez Khushk*) belongs to the *Umbelliferae* family. It is referred to as coriander in English, *Koththamalli* in Tamil, and *Kottamalli* in Sinhala. The plant contains several bioactive compounds, including coriander oil, p-cymol, geraniol, pentosan, furfurol, pectin, coumarins, flavonoids, phenolic acids, vitamin C, fat, protein, starch, and potassium malate [7]. *Khasneez Khushk* possesses multiple therapeutic properties, including *Musakkin* (sedative), *Muqawwi e Dimagh* (brain stimulant), and *Muqawwi e Qalb* (heart stimulant). These actions make it beneficial in managing Generalized Anxiety Disorder (GAD) [1]. Additionally, *Khasneez Khushk* is recognized for its *Muqawwi* (stimulant), *Kasurriyah* (carminative), *Muqawwi e Meda* (stomachic), tonic, antibacterial, antioxidant, antispasmodic, diuretic, diaphoretic, nervine, and aphrodisiac properties [8]. *Khasneez Khushk* is commonly used to treat conditions such as fever, diarrhea, indigestion, piles, vomiting, cough, and asthma in children [9]. The fruit of this herb is widely used in combination with dry ginger as a decoction for colds, influenza, and fever. An infusion of the fruit is often prescribed for dyspepsia, sore throat, catarrh, and bilious complaints. The oil of *Khasneez Khushk* is effective for treating flatulent colic, rheumatism, and neuralgia [7]. Additionally, it is used to improve eyesight [10].

Piper nigrum (commonly known as *Filfil-e-Siyah*) belongs to the *Piperaceae* family. It is referred to as black pepper in English, *Milagu* in Tamil, and *Gammiris* in Sinhala [7]. The major bioactive components of *Filfil-e-Siyah* include piperine, piperazine, acrid resin, oleoresin, volatile oil, and chavicine, among others. *Filfil-e-Siyah* is renowned for its carminative, stomachic, and antacid properties. It is also used as an antiperiodic, rubefacient, and counter-irritant when applied externally to the skin. Additionally, it possesses antipyretic, antidiarrheal, anti-dysenteric, and analgesic effects. *Filfil-e-Siyah* is commonly utilized in the management of asthmatic conditions [11] and is recognized for its antibacterial, mucoid, antioxidant, antiseptic, and aromatic properties [8].

2. Materials and Methods

2.1 Procurement of Raw Drugs

The ingredients of Safoof-e-Musakkin were sourced from the local market. The authentication of these raw materials was carried out by the Department of Dravyaguna and Swasthivritta, Faculty of Indigenous Medicine, University of Colombo. A detailed list of the ingredients is provided in Table 2.

Table 2: Details of the Ingredients

No	Name	Botanical name	Part used	Proportion
1	*Beekh e asrol*	*Rauvolfia serpentina*	Root	44.64%
2	*Kasneez khushk*	*Coriandrum sativum*	Seed	44.64%
3	*Filfil-e-siyah*	*Piper nigrum*	Fruit	10.71%

Fig 1: Safoof-e-Musakkin

2.2 Preparation of Formulation

The ingredients were thoroughly cleaned and shade-dried before being powdered by passing them through sieve #80. Safoof-e-Musakkin was then prepared following the method outlined in the National Formulary of Unani Medicine.

2.3 Organoleptic Evaluation

The Safoof-e-Musakkin formulation was evaluated organoleptically based on its color, odor, taste, appearance, particle size, and texture.

2.4 Physicochemical Evaluation

The physicochemical evaluation of Safoof-e-Musakkin was conducted by assessing the loss of weight on drying at 105°C, total ash, water-soluble ash, and acid-insoluble ash. These tests were performed according to the protocols outlined for Ayurveda, Siddha, and Unani medicines, as well as the guidelines provided in the Unani Pharmacopeia of India [12, 13].

(a) Determination of Total Ash: An accurately weighed sample (2-4 g) of the extract was placed in a crucible. The sample was spread evenly and ignited to a constant weight by gradually increasing the temperature to 500-600 °C.

(b) Determination of Water-Soluble Ash: To the crucible containing the total ashes, 25 mL of water was added, and the mixture was boiled for 5 minutes. The water-insoluble matter was then collected on an ashless filter paper and washed with hot water. The filter paper containing the water-insoluble matter was transferred back to the original crucible, dried on a hot plate, and ignited to a constant weight. The weight of the residue was subtracted from the weight of the total ash, and the content of the water-soluble ash was calculated.

(c) Determination of Acid-Insoluble Ash: To the crucible containing the total ash, 25 mL of 2M hydrochloric acid (HCl) was added. The mixture was covered with a watch glass and gently boiled for 5 minutes. The watch glass was then rinsed with 5 mL of hot water, and the rinsed contents were added to the crucible. The acid-insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the acid-insoluble matter was transferred back to the original crucible, dried on a hot plate, and ignited to a constant weight.

2.5 Phytochemical Evaluation

The qualitative phytochemical analysis of Safoof-e-Musakkin was conducted to identify the presence or absence of various chemical constituents, including tannins, flavonoids, terpenoids, alkaloids, phenols, carbohydrates, saponins, steroids, and reduce sugars.

Sample Preparation: The plant materials were extracted using hot water extraction. The extract was then filtered through cotton wool and used for the phytochemical screening.

2.6 Test for Saponins

Five milliliters of the extract and 2.5 mL of water were added to a test tube and shaken vigorously. The presence of stable froth indicates the presence of saponins.

2.7 Tests for Tannins

(a) Ferric Chloride Test: Five drops of ferric chloride ($FeCl_3$) were added to the extract and mixed well. The appearance of a black precipitate indicates the presence of tannins.

(b) Lead Acetate Test: Three drops of lead acetate ($Pb(OAc)_2$) were added to 5 mL of the extract and mixed. The formation of a yellow precipitate suggests the presence of tannins.

(c) Vanillin Test: A few drops of 10% vanillin in ethyl alcohol and concentrated hydrochloric acid (HCl) were added to the extract. The appearance of a red color indicates the presence of tannins.

2.8 Test for Phenols

(a) Lead Acetate Test: Three drops of lead acetate ($Pb(OAc)_2$) were added to 5 mL of the extract. The formation of a yellow precipitate indicates the presence of phenols.

(b) Vanillin Test: A few drops of 10% vanillin in ethyl alcohol and concentrated HCl were added to 2 mL of the extract. The appearance of a red color indicates the presence of phenols.

2.9 Test for Alkaloids

Wagner's Reagent Test: Two drops of Wagner's reagent were added to 2 ml of the extract and mixed. The appearance of a reddish color indicates the presence of alkaloids.

2.10 Test for Flavonoids

(a) Five milliliters of dilute ammonia solution were added to 5 ml of the extract, followed by the addition of concentrated H_2SO_4. The appearance of a yellow color indicates the presence of flavonoids.

(b) Five milliliters of the extract were added to a test tube containing a piece of metallic magnesium and 3 drops of concentrated HCl and heated. A red-orange color indicates the presence of flavonoids.

2.11 Test for Terpenoids

(a) Salkowski Test: Five milliliters of the extract were mixed with 2 ml of chloroform in a test tube, and 3 ml of concentrated H_2SO_4 was added along the sides of the test tube. The formation of a reddish-brown color indicates the presence of terpenoids.

(b) Test for Sesquiterpenes: One milliliter of concentrated H_2SO_4 was added to 2 ml of the extract. A reddish-brown color indicates the presence of sesquiterpenes.

2.12 Test for Steroids

(a) Five milliliters of acetic anhydride and 5 ml of concentrated H_2SO_4 were added to 5 ml of the extract and mixed. A colour change from violet to blue or green indicates the presence of steroids.

(b) Liebermann-Burchard Test: Two milliliters of acetic anhydride and 2 ml of concentrated H_2SO_4 were added to 2 ml of the extract and mixed. The formation of a dark bluish-green color indicates the presence of steroids.

2.13 Test for Carbohydrates

Two to three drops of iodine solution were added to the extract. The appearance of a blue color indicates the presence of starch.

2.14 Test for Proteins

A few drops of biuret reagent were added to the extract. The appearance of a violet color indicates the presence of proteins.

2.15 Test for Reducing Sugars

0.5 mL of each of Fehling's reagents A and B were added to a few milliliters of the extract. The test tubes were placed in a water bath for 1-2 minutes. The appearance of a brick-red color indicates the presence of reducing sugars.

2.16 TLC Fingerprinting Analysis

(a) Extraction Method: The formulation was extracted using dichloromethane (DCM), concentrated, and then spotted onto a pre-coated TLC plate.

(b) Solvent System: The mobile phase consisted of a mixture of dichloromethane, ethyl acetate, and cyclohexane in a ratio of 5:2:5 (v/v).

(c) Spray Reagent: Vanillin-sulphate reagent was used for visualization, and the plate was heated at 110 °C for 5 minutes. [14]

3. Results and Discussion

Neurological disorders are an increasing concern in the modern industrialized world, with a significant burden on global health. [15] According to a World Health Organization (WHO) report, neurological disorders affect up to one billion people worldwide. [16] Safoof-e-Musakkin has been identified as a potential treatment for various neurological disorders, including anxiety, headaches, and delirium.[17] While herbal formulations have traditionally been considered safe, their increasing use without proper guidance can lead to issues of efficacy and safety. Some herbal preparations may be ineffective or even cause health complications. Therefore, standardizing herbal medicines is crucial to evaluate the quality, purity, and concentration of their active chemical constituents. [18] Standardization is an essential process for determining the quality, purity, and identification of herbal products, ensuring they meet the required specifications for therapeutic use.

The organoleptic properties of Safoof-e-Musakkin were characterized by a light brown color, a characteristic odor, a bitter taste, moderately fine texture, and a particle size corresponding to sieve #80. The powder characteristics of Safoof-e-Musakkin are presented in Table 3.

Table 3: Organoleptic evaluation of Safoof-e-Musakkin

Color	Light brown color
Odor	Characteristic odor
Taste	Bitter taste
Texture	Moderately fine texture

Table 4 provides the results of the physicochemical evaluation of Safoof-e-Musakkin. The average weight loss upon drying for Safoof-e-Musakkin was 7.1 ± 0.6%. Plant drugs typically contain between 8% and 14% moisture, and excessive moisture can lead to hydrolysis, bacterial or fungal growth, and biochemical degradation.[19] The low moisture content in Safoof-e-Musakkin suggests it is stable and secure for long-term storage. The total ash value, an essential factor in the quality assessment of herbal medications, provides insights into contamination or impurities. A high ash value may indicate the presence of foreign materials or improper preparation methods. The total ash content of Safoof-e-Musakkin was 12.5± 0.3%, which is relatively low, indicating minimal contamination. Water-soluble ash, which serves as an indicator of improperly prepared medicines or the extraction of water-soluble salts, was found to be 3.6 ± 0.4%. Additionally, the acid-insoluble ash value was less than 0.2%. [20]

Table 4: Physicochemical evaluation of Safoof-e-Musakkin

Parameters	Percentage
Loss on drying at 105 c	7.1 +_ 0.6%
Total ash	12.5 +_ 0.3%
Water soluble ash	3.6 +_ 0.4%
Acid insoluble ash	Less than 0.2%

Phytochemical analysis of Safoof-e-Musakkin revealed the presence of tannins, phenols, flavonoids, terpenoids, saponins, carbohydrates, and reducing sugars. These bioactive compounds have been reported to exhibit significant pharmacological effects. For example, terpenes, flavonoids, and tannins are known to have antidepressant properties. Various saponins and triterpenoids have been identified as bioactive substances with therapeutic potential for psychiatric disorders [21].

Tannins have neuroprotective, antioxidant, anti-inflammatory, anti-apoptotic, anti-bacterial, anti-aging, and anti-hyperglycemic properties. These properties are particularly valuable in defending against neurotoxins and oxidative stress-induced neuronal damage, making tannins critical in neuroprotection [22]. Similarly, phenols exhibit antioxidant and radical-scavenging properties, offering protection to nerve and glial cells and contributing to neuroprotection [23]. Flavonoids are known for their ability to protect against oxidative stress, which is believed to be a contributing factor in many central nervous system disorders [24].

Terpenoids have shown efficacy in treating psychiatric conditions and possess neuroprotective properties, including memory enhancement, antioxidant activity, and antidepressant and anxiolytic effects [25]. Saponins have been shown to regulate neurotransmitters, inhibit tau phosphorylation, and promote the regeneration of neural networks, thus offering potential therapeutic benefits for central nervous system disorders [26]. Carbohydrates play a key role in improving cognitive function by regulating energy supply to the brain, reducing stress, and contributing to neuron development and myelin formation, making them important in treating developmental abnormalities and mental retardation [27]. The presence of these phytochemicals in Safoof-e-Musakkin underscores the formulation's potential to treat neurological disorders effectively.

The phytochemical screening results are summarized in Table 5, while Figures 2, 3, and 4 illustrate the presence of tannins, saponins, and carbohydrates, respectively.

Table 5: Phytochemical screening of Safoof-e-Musakkin

No.	Type of phytochemical	Test	Result
1.	Tannins	Ferric chloride test	++
		Lead acetate test	+
		Vanillin test	-
2.	Phenols	Lead acetate test	+
		Vanillin test	-
3.	Flavonoids	Dil ammonia+conc sulfuric acid	+
		Zn+conc HCl	+
4.	Alkaloids	Wagner reagent	-
5.	Terpenoids	Salkowski test	+
		Test for sesquiterpenes	+
6.	Steroids	Acetic anhydride +Conc sulfuric acid	-
		Liberman Burchard test	-
7.	Saponins	Froth test	+
8.	Carbohydrates	Iodine test	++
9.	Proteins	Biuret test	-
10.	Reducing Sugars	Fehlings test	+

-ve: negative, +: positive in low level, ++: Positive in moderate level

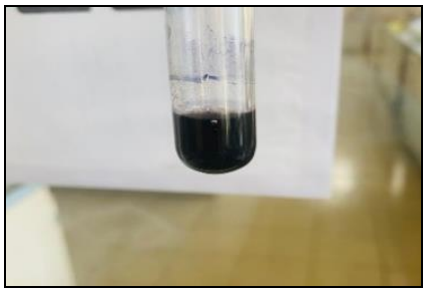

Fig 2: Saponins present

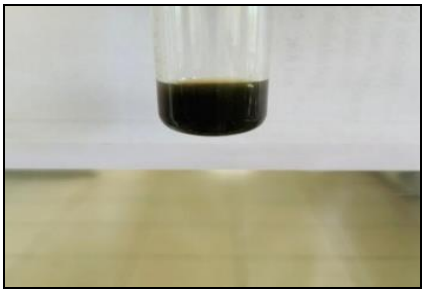

Fig 3: Tannin present

Fig 4: Carbohydrate present

TLC fingerprinting analysis was performed on Safoof-e-Musakkin under 254 nm and 366 nm UV light. The fingerprinting data, obtained using the specified solvent system, will aid in the authentication and identification of Safoof-e-Musakkin. Table 6 presents the TLC screening data, and Figures 5 and 6 show the TLC fingerprint profiles before the application of Vanillin sulfate at 254 nm and 366 nm. Figure 7 displays the TLC fingerprint profile after the application of Vanillin sulfate.

Table 6: TLC Techniques

R_f values and Colours of the Sample		
Before Spraying: 254 nm and 366 nm	**After spraying Vanillin sulphate**	
0.02	0.02	Pink
0.15	0.08	Pink
0.25	0.20	Grey
0.40	0.25	Pink
0.45	0.40	Pink
0.58	0.59	Purple
0.74	0.75	Purple
0.82	0.79	Pink
0.85	0.85	Brownish Red
	0.90	Brownish Red

Fig 5: TLC Fingerprint profile at 254 nm

Fig 6: TLC Fingerprint profile at 366 nm

Fig 7: TLC Fingerprint after spraying Vanillin sulphate

The pharmacological activities of the ingredients used in Safoof-e-Musakkin were reviewed from the literature. *Rauvolfia serpentina* (*Beekh-e-Asrol*) plays a significant role in neuropharmacology by affecting chemical transmission in the central nervous system. It has sedative, hypnotic, and serotonin-releasing properties, and has been used to treat mental disorders, psychosis, and hypertension [28]. *Coriandrum sativum* (*Kasneez Khushk*) is known for its antianxiety, anticonvulsant, and memory-enhancing properties, as well as its neuroprotective effects [17]. *Piper nigrum* (*Filfil-e-Siyah*) has been shown to have antidepressant, anti-epileptic, neurodegenerative protection, and antioxidant properties [29, 30].

These pharmacological properties align with the phytochemical components detected in Safoof-e-Musakkin, supporting its potential use in the treatment of neurological

disorders. The standardization established in this study serves as an initial reference for the formulation, and more advanced standards can be developed in the future through quantitative analysis of its constituents.

4. Conclusion

The prepared powder composition of Safoof-e-Musakkin, formulated in accordance with Unani pharmacopeial standards, was subjected to a comprehensive evaluation of various standardization parameters. Phytochemical analysis, including Thin Layer Chromatography (TLC), was conducted to establish its quality standards. The findings from these standardization processes can serve as a reliable standard monograph for the recognition, further assessment, and potential future research on Safoof-e-Musakkin.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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